

DATA ACCEPTABILITY CRITERIA FOR TOXICITY TESTING SAMPLES

Test Organism (Protocol – MPSL SOP)	Acceptability Criteria
<i>Ampelisca abdita</i> (10-day) (US EPA 2000 – MPSL SOP 2.2)	a) Survival in the controls $\geq 85\%$. b) All performance criteria outlined in SOP are met.
<i>Atherinops affinis</i> (7-day) (US EPA 1995 – MPSL SOP 2.15)	a) Survival in the controls $\geq 80\%$. b) 0.85 mg average weight of control larvae (9 days old). c) Copper $LC_{50} \leq 205 \mu\text{g/L}$. d) $< 25\%$ MSD for survival and $< 50\%$ MSD for growth. e) All performance criteria outlined in SOP are met.
<i>Ceriodaphnia dubia</i> (acute 96-hour) (US EPA 1993 – MPSL SOP 2.4)	a) Survival in the controls $\geq 90\%$. b) All performance criteria outlined in SOP are met.
<i>Ceriodaphnia dubia</i> (chronic 7-day) (US EPA 1994 – MPSL SOP 2.3)	a) Survival in the controls $\geq 80\%$. b) Surviving females: average 15 neonates c) Surviving females: 60% have 3 or more broods d) All performance criteria outlined in SOP are met.
<i>Eohaustorius estuarius</i> (10-day) (US EPA 2000 – MPSL SOP 2.5)	a) Survival in the controls $\geq 90\%$. b) All performance criteria outlined in SOP are met.
<i>Haliotis rufescens</i> (48-hour) (US EPA 1995 – MPSL SOP 2.1)	a) Normal shell development in the controls $\geq 80\%$. b) Statistical significant effect at $56 \mu\text{g/L}$ zinc. c) $< 20\%$ MSD. d) All performance criteria outlined in SOP are met.
<i>Holmesimysis costata</i> (7-day) (US EPA 1995 – MPSL SOP 2.6)	a) Survival in the controls $\geq 75\%$. b) $> 0.4 \mu\text{g}$ average dry weight in controls. c) Survival and growth NOECs $\leq 100 \mu\text{g/L}$ Zn. d) $< 40\%$ MSD for survival and $< 50 \mu\text{g}$ MSD for growth. e) All performance criteria outlined in SOP are met.
<i>Hyalella azteca</i> (10 and 28-day) (US EPA 2000 – MPSL SOP 2.7)	a) Survival in the controls must be $\geq 80\%$. b) Measurable growth in controls. c) All performance criteria outlined in SOP are met.

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<i>Macrocystis pyrifera</i> (48-hour) (US EPA 1995 – MPSL SOP 2.8)	<ul style="list-style-type: none"> a) Germination in the controls $\geq 70\%$. b) $>10\ \mu\text{m}$ germ-tube length in controls. c) NOECs $<35\ \mu\text{g/L}$ Cu. d) $<20\%$ MSD for germination and growth in reference toxicant. e) All performance criteria outlined in SOP are met.
<i>Mytilus galloprovincialis</i> (48-hour) (US EPA 1995 – MPSL SOP 2.9)	<ul style="list-style-type: none"> a) Control normal survival $\geq 70\%$ (or with two endpoints: survival $\geq 50\%$ and normal development $\geq 90\%$). b) $<25\%$ MSD. c) All performance criteria outlined in SOP are met.
<i>Pimephales promelas</i> (chronic 7-day) (US EPA 1994 – MPSL SOP 2.10)	<ul style="list-style-type: none"> a) Survival in the controls $\geq 80\%$. b) $>0.25\ \text{mg}$ average weight of control larvae. c) All performance criteria outlined in SOP are met.
<i>Selenastrum capricornutum</i> (96-hour) (US EPA 1994 – MPSL SOP 2.11)	<ul style="list-style-type: none"> a) $>200,000$ cells/mL without EDTA ($1,000,000$ cells/mL with EDTA). b) Variability of controls $<20\%$. c) All performance criteria outlined in SOP are met.
<i>Strongylocentrotus purpuratus</i> (20-minute) (US EPA 1995 – MPSL SOP 2.16)	<ul style="list-style-type: none"> a) Fertilization in the controls $\geq 70\%$. b) $< 25\%$ MSD. c) All performance criteria outlined in SOP are met.
<i>Strongylocentrotus purpuratus</i> (96-hour) (US EPA 1995 – MPSL SOP 2.14)	<ul style="list-style-type: none"> a) Normal shell development in the controls $\geq 80\%$. b) $< 25\%$ MSD. c) All performance criteria outlined in SOP are met.

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TIE Acceptability Criteria

Treatments from toxicity identification evaluations are considered acceptable if the organism response in the treatment blank is not significantly different from the baseline treatment control.

ELISA Acceptability Criteria

To verify accuracy of the ELISA method, an external standard is quantified during every field survey. The external standard is made from reagent-grade chemical spiked into Nanopure® water at a known concentration. Accuracy of these measurements is considered acceptable if the measured value is within 20% of the nominal concentration. To assess matrix interference, a matrix spike is quantified for every 20 field samples analyzed. The matrix spike consists of the external standard spiked into river water. Precision of the ELISA method is determined by calculating the coefficient of variation of duplicate measurements of a field sample. CVs less than 20% for duplicate field samples are considered acceptable.

At least 5% of the samples measured with ELISA kits are also measured with EPA analytical chemistry methods for comparison. Differences between the analyses are quantified as the Relative Percent Difference (RPD) for both chlorpyrifos and diazinon. If the RPD of the two analytical methods is greater than 50% for any sample, results of the quality assurance measures are evaluated to determine any deviations from QA guidelines, and both values are reported. In these situations the toxicology data are also evaluated to see if these data suggest the presence of the analyte at toxic concentrations. The toxicity test data and any available TIE data are compared to both the ELISA and instrumental analyses in a weight-of-evidence approach to evaluate the significance of the chemical analyses in the context of the toxicity test results.

Other Quality Assurance Criteria for Toxicity Testing

Precision Criteria for Toxicity Tests and Water Quality Measurements

Field duplicates: The precision of sample toxicity determinations is estimated through the analysis of field duplicate samples. In the SWAMP Program, field duplicates are collected at a frequency of 5% per sampling event/trip (if less than 20 samples collected, one field duplicate is collected). Field duplicates are tested side-by-side with an original sample to estimate variability associated with sampling and laboratory procedures. Each duplicate sample is collected immediately after collecting the corresponding test sample, and is handled and tested in the same manner. Test results from the original sample and its duplicate are compared to determine coefficients of variation as indicators of test precision. The coefficient of variation (CV) is calculated as the standard deviation of the two sample values divided by the mean (x 100). The CVs are reported as the mean and range of CVs for all samples and duplicate samples tested. Although there is no acceptable CV for toxicity testing of duplicate samples for precision estimations, these may be compared to similar values reported by the Marine Pollution Studies Laboratory for previous and continuing SWRCB monitoring studies.

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Positive and negative controls: Each toxicity test will include positive and negative controls.

Positive-control reference toxicant tests will be conducted monthly for organisms produced in controlled cultures, and concurrently with each sample test using organisms collected from the wild..

- Each *Ceriodaphnia* reference toxicant test will consist of a dilution series of 0, 5.6, 10, 18, 32 and 56 µg/L copper (from cupric chloride).
- Each *Hyaella* reference toxicant test will run for four days and consist of a dilution series of 0, 5, 10, 20 and 40 µg/L cadmium (from cadmium chloride).
- Three replicates of each concentration will be tested, with 10 animals per replicate.
- Any tests in which reference toxicant test LC₅₀ values are out of control limits of 2 standard deviations of the MPSL mean value are reported to the QA Officer and Contract Manager.
- Reference toxicant test control chart variations are noted in any interpretation of study data.

Negative controls will be conducted with each batch of toxicity test samples.

- Two negative controls for *Ceriodaphnia* tests will consist of laboratory dilution water (4:1 distilled or Nanopure® water to Evian® water) adjusted with seawater to the lowest and highest conductivity observed in the set of test samples.
- The negative control for *Hyaella* solid phase tests will consist of reference sediment subjected to the same well-water renewals as the samples. Control replicates will be interspersed randomly among test replicates, using the same number of test organisms, feeding rates, and renewal schedules, as for the test samples.

Laboratory general water quality measurements

- **Dissolved oxygen, pH, conductivity, and ammonia:** The precision of dissolved oxygen, pH, conductivity, and ammonia measurements will be determined by measuring a standard at the beginning and end of each set of 10 sample measurements. Precision of these measurements will be given as the coefficient of variation, which will be reported if it exceeds 10% for dissolved oxygen, pH, and conductivity, and 30% for ammonia.
- **Temperature:** Temperature will be recorded continuously, and temperatures >1° C above or below target test temperature will be reported.
- **Hardness and alkalinity:** Precision of hardness and alkalinity measurements will be determined by measuring standards on a quarterly basis. Precision of these measurements will be given as the coefficient of variation, which will be reported if it exceeds 10%.

Accuracy Criteria for Toxicity Test Water Quality Measurements

Accuracy criteria are not applicable to toxicity testing endpoints, because there are no standard organism responses against which to compare test results. In place of an absolute measurement of accuracy for toxicity tests, reference toxicant tests are

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conducted to determine whether organism response is within prescribed control limits, as described above.

Accuracy of dissolved oxygen, pH, conductivity, and ammonia measurements is assessed by measuring standard solutions at the beginning and end of each set of 10 measurements. Each measured value of a standard is compared against the known value of the standard, and accuracy is expressed as the relative percent difference. Accuracy of water quality measurements is calculated as follows:

$$RPD = \frac{[V_m - V_k]}{V_k} \times 100\%$$

Where: RPD = the relative percent difference

V_m = the measured value,

V_k = the known value.

If an RPD value for dissolved oxygen, pH, or conductivity exceeds 10%, the measurements conducted since the previous accuracy check will be repeated. If an RPD value for a total ammonia measurement exceeds 30%, the measurements conducted since the previous accuracy check will be repeated.

Accuracy of hardness and alkalinity measurements is assessed by measuring standards on a quarterly basis. Each measured value of a standard is compared against the known value of the standard, and accuracy is expressed as the relative percent difference (see calculation of RPD, above). RPD values above 10% are reported.

Completeness Criteria for Toxicity Testing

The SWAMP Program has as an overall goal of 90% completeness for all tests and analyses undertaken.

Representativeness Criteria for Toxicity Testing

The EPA Technical Support Document (EPA 1991) summarizes several studies that support the use of EPA's freshwater testing protocols. *Ceriodaphnia* are generally considered appropriate surrogates for native species of crustacea, which form important links in food webs leading to higher trophic level organisms such as fish, amphibians and waterfowl. *Ceriodaphnia* are known to be sensitive to pesticides, industrial chemicals, some metals, and other compounds that might affect wildlife in the State's surface waters. *Hyalella* is also sensitive to pesticides, and is a resident species in many of the State's surface waters.

Toxicity test results are considered representative of acute toxicity of the water sample if the sampling protocol is followed, tests are initiated within 48 hours of sample collection, and laboratory water chemistry results are within the ranges observed in the field.

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Comparability Criteria for Toxicity Testing

By strictly following MPSL toxicity test SOP's, documenting test conditions, and comparing results from reference toxicant tests, this project will produce results that can be quantitatively compared to results obtained by other laboratories conducting similar analyses. The *Ceriodaphnia* test is commonly used in surface water assessments (DeVlaming *et al.* 2000), and use of this method will allow the results of these toxicity tests to be compared to those of other water bodies. The EPA protocol for *Hyalella* (EPA 2000) is used in numerous programs across the nation, and the toxicity of SWAMP samples can be compared to the toxicity of numerous other samples collected nationwide. This level of comparability also exists with most of the toxicity testing protocols described above for use in the SWAMP program.